

Appendix Q
Colonization of Cargo Residue in the Great
Lakes by Zebra Mussel (*Dreissena polymorpha*)
and Quagga Mussel (*Dreissena bugensis*)

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Introduction

The U.S. Coast Guard is preparing an EIS to support rule making for management of dry cargo residue (DCR). Concern over DCR discharged to the lakes as potential substrate for the colonization of the invasive species *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) within the Great Lakes has prompted an investigation into their attachment onto these residues. Invasion of the Great Lakes by dreissenids has caused both environmental and economic concerns. Providing additional habitat for their proliferation may increase their expansion into other areas of the lakes. This technical memorandum consists of a literature review and provides input in the EIS analysis of invasive mussel impacts in the lakes. The goals of the literature review are the following:

- Discuss life processes of *Dreissena spp*
- Document limiting factors of *Dreissena spp*, particularly substrate preferences
- Consider ecological and economic impacts of *Dreissena spp* colonization
- Find and interpret relatively recent *Dreissena polymorpha* (zebra mussel) and *Dreissena bugensis* (quagga mussel) distributions in the open waters of the Great Lakes in relation to navigational track lines of cargo ships

Origin

Zebra mussels are considered native to the Black Sea, Caspian Sea, and Ural River areas of Eurasia. Quagga mussels are indigenous to the Dneiper River drainage of Ukraine and were reported in Ukraine's Bug River in 1890 (Andrusov, 1890). Canals built in Europe have allowed both of these species to expand their ranges, and they now have expanded into most major drainages in Europe. Zebra and quagga mussels in the Great Lakes have been introduced by numerous sources in northwestern and north central Europe, from which shipping to the Great Lakes originates (Jentes, 2001). Zebra mussels were first discovered in Lake St. Clair in 1988 and quagga mussels were first noted in Lake Erie in 1989. Quagga mussels were not identified as a distinct species until 1991.

Life Processes

Reproduction and Development

Both zebra and quagga mussels are prolific breeders; this possibly contributes to their spread and abundance. *Dreissena spp* are dioecious (either male or female) with external fertilization. A fully mature female mussel is capable of producing up to one million eggs per season. Reproduction of zebra mussels usually occurs in the spring or summer, depending on water temperature. Optimal temperature for spawning is 14°C to 16°C (USGS, 2005); in waters that are warm throughout the year, spawning may occur over longer periods. Spawning for quagga mussels in profundal areas is reported to occur at 9°C (Claxton and Mackie, 1998). This lower spawning temperature may give the quagga mussel an advantage over the zebra mussel and may contribute to its invasions in the northern Great Lakes.

Dreissenid early life history evolves through the veliger, post-veliger, and adult stages. The veligers are photopositive, active swimmers using a ciliated velum (derived from the prototroch of the trocophore larva). After 10–15 days, the veligers metamorphose to the first post-veliger stage, the pediveliger. The pediveliger becomes photonegative and settles to the benthos in search for a suitable substrate for attachment. The pediveliger has a velum and a ciliated foot and uses both in substrate exploration. It is the pediveliger that is the primary life stage involved in substrate selection. Once the development proceeds to the next post-veliger stage, the plantigrade, it loses its velum and can no longer swim. Once in contact with the substrate, the post-veliger attaches and completes shell development and maturation to an adult.

Dispersion Processes

Zebra and quagga mussels are dispersed by a variety of mechanisms. Generally, in the presettling stage, mussel veligers are moved by prevailing water currents. As post-veligers become photonegative, settling down the water column, they drift with currents until they encounter a suitable attachment surface.

Mussels attach to surfaces by secreting a tuft of fibers known as byssal threads (collectively forming a bysuss) from a gland near the foot of their shells. The threads have an adhesive disk at the end that attaches to surfaces by secreting a protein adhesive. To detach, the mussels secrete enzymes that break the byssal threads near the foot. Byssal threads are regenerated after detachment (Claudi and Mackie, 1994).

Adult mussels can relocate either by crawling, which can occur at rates up to several meters per day, or by moving with currents after detachment (Maryland Sea Grant, 1994). Adults will reposition themselves to a more advantageous location to obtain food. Translocation of adult mussels is most common in fall and winter months (Claudi and Mackie, 1994). To a lesser extent, waterfowl and other aquatic organisms also assist in the dispersal of these mussels.

Feeding

Both mussels are filter feeders; they use their cilia to pull water into their shell cavity where it passes through an incurrent siphon and desirable particulate matter is removed. Each adult mussel is capable of filtering one or more liters of water each day and removing

phytoplankton, zooplankton, and even their own veligers (Snyder et al., 1997; USGS, 2007a). Any undesirable particulate matter is bound with mucus, known as pseudofeces, and ejected out the incurrent siphon. The particle-free water is then discharged out the excurrent siphon.

Natural Predators

European populations of diving ducks have changed their migration patterns in order to forage on beds of zebra mussels (Molloy et al., 1997). This most extreme case occurred on Germany's Rhine River. Overwintering diving ducks and coots consumed up to 97 percent of the standing crop of mussels each year. However, high mussel reproduction rates replenished the population each summer. Molloy et al. (1997) cited 176 species involved in predation, 34 in parasitism, and 10 in competition with mussels.

In North America, the species most likely to prey on relatively deep beds of zebra and quagga mussels are scaup, canvasbacks, and oldsquaws. But populations of these species are quite low; in the Great Lakes, diving ducks are migrating visitors, pausing only to feed during migrations. However, Canadian researchers have documented increasing numbers of migrating ducks feeding on zebra mussels around Point Pelee in western Lake Erie. In southern Lake Michigan, zebra mussels encrusting an underwater power plant intake attracted flocks of lesser scaup. Unfortunately, some were pulled into the intake pipe and drowned. The stomachs of these dead scaup were full of zebra mussels. Mallard ducks also are frequently observed foraging on zebra mussels on shoreline rocks and shallow structures. Additionally, round goby (*Neogobius melanostomus*) and freshwater drum (*Aplodinotus grunniens*) are known to feed substantially on *Dreissena* spp (French and Love, 1995; Walsh et al., 2007). While drums may reduce population, they are not an effective biological controller because of feeding limitations based on mussel shell size (French and Love, 1995). Yellow perch (*Perca flavescens*) have been observed feeding on juveniles, particularly when they are detached and drifting.

Limiting Factors

Although zebra and quagga mussels are similar species, limiting factors vary slightly, as shown in Table 1.

Food Supply

Food availability is one of the most essential factors for *Dreissena* spp growth (Chase and Bailey, 1999). Insufficient food can compromise the structure of *Dreissena* spp byssal threads and lead to weak attachment (Clarke, 1999). Total suspended solids and phytoplankton represent the *Dreissena* spp food sources (USGS, 2007a). In Lake Huron, zebra mussel growth was affected nine times more by phytoplankton biomass (measured by chlorophyll-*a*) than by temperature (Chakraborti et al., 2002). As expected, higher nutritional quality of food aids reproduction success by increasing mussel egg mass (Wacker and Elert, 2003).

TABLE 1
Environmental Requirements for Great Lakes Invasive Mussels

Parameter	Zebra	Quagga	Reference
Preferred temperature (°C)	10–25	As low as 5	Karatayev et al. (1998), Paukstis et al. (1997), Roe and MacIsaac (1997), Claudi and Mackie (1994)
Preferred calcium level (mg/L)	44–50	Perhaps higher than for zebra mussels	Sprung (1987), Jones and Ricciardi (2005)
Preferred pH	7.4–9.3	Presumed similar to zebra mussels	Sprung (1987), Bowman and Baily (1998)
Preferred DO (% saturation)	At least 25	Perhaps lower than for zebra mussels	Karatayev et al. (1998)
Preferred depth (ft)	15–25	Up to at least 300	Mills et al. (1993, 1999), Egan (2006)
Reported extreme depths (ft)	360, Lake Ontario	540, Lake Michigan	Mills et al. (1993), Egan (2006)

Note: DO, dissolved oxygen.

Temperature

Temperature is another major factor in zebra mussel survival and reproduction (Chase and Bailey, 1999; Wacker and Elert, 2003). Zebra mussel survival temperatures range from 0°C to slightly in excess of 30°C for short periods; optimum temperatures are generally less than 25°C (Paukstis et al., 1997). The minimal temperature for growth and development is approximately 10°C (Karatayev et al., 1998). Increased temperatures usually increase feeding rates. Zebra mussel spawning (release of gametes into the water column) will generally not occur at temperatures below about 12°C (Claudi and Mackie, 1994).

Quagga mussels have been found in temperatures less than 5°C in Lake Ontario and there is evidence that quagga mussels are capable of spawning at temperatures near 5°C (Mills et al., 1993; Roe and MacIsaac, 1997). This may give them an advantage over the zebra mussel and account for their proliferating in the hypolimnion of the some Great Lakes. Claxton and Mackie (1998) found that quagga mussels spawned between 9°C and 10°C whereas zebra mussels neither spawned nor showed significant gametogenic development at these temperatures. MacIsaac (1994) reported that high water temperature in the Great Lakes would not likely affect quagga mussel distribution.

Calcium Level

The significance of calcium as a limiting factor for zebra mussels depends on the life stage of the mussel. Although adult zebra mussels can tolerate low-calcium waters, veligers are most successfully reared within a calcium level ranging from 44 to 50 mg/L, with minimum range of 12–24 mg/L (Sprung, 1987; Ram and Walker, 1993). Because veligers are highly sensitive to calcium, calcium is a critical characteristic for zebra mussel population establishment. Zebra mussels do not survive when there is prolonged low-calcium concentration in the water because calcium is an essential element in the composition of the bivalve shell. Calcium concentrations of 15 mg/L or less were found to limit the distribution of zebra mussels in the St. Lawrence River (Mellina and Rasmussen, 1994). Laboratory-

based studies conducted by Hincks and Mackie (1997) reported maximum growth at 32 mg/L and negative shell growth at 8.5 mg/L. Jones and Ricciardi (2005) indicated that zebra mussel populations occurred at calcium levels as low as 8 mg/L.

Quagga mussels were found to be absent below calcium concentrations of 12 mg/L, which suggests that they may have a higher calcium requirement than the zebra mussel (Jones and Ricciardi, 2005).

pH

The amount of hydrogen ions in the water – that is, pH – is critical in determining whether zebra mussels will be able to survive and reproduce in a water body. A pH of 7.4 or less inhibits larval development (Sprung, 1987). Laboratory-based studies conducted by Bowman and Baily (1998) indicated an upper tolerance limit of between 9.3 and 9.6. Hincks and Mackie (1997) reported that positive growth in juvenile zebra mussels occurred only at a pH greater than 8.3. Despite the general threshold, in laboratory studies Mikheev (1964) found that water with a pH of 6.6 and a calcium level less than 12 mg/L could host a mussel population greater than 500,000/m². This has not been documented in the field.

Information on the effects of pH on the quagga mussel is lacking, but the effects would likely be similar to those on the zebra mussel.

Dissolved Oxygen Level

In 1992, Lake Erie's area with periodic summer anoxia was the only region of the basin that was not colonized with *Dreissena spp* (Dermott and Munawar, 1993). This observation strongly suggests that dissolved oxygen is a limiting factor to population density and occurrence. Successful growth and reproduction of zebra mussels requires at least 25 percent oxygen saturation (Karatayev et al., 1998). Due to their preferred shallow water habitat, this usually is not a problem. Although zebra mussels can survive at very low concentrations for short periods of time, growth and reproduction will be limited (Woyanovich, 1961).

As with pH, there is little information on dissolved oxygen requirements for the quagga mussel. Based on its ability to colonize deeper areas of the Great Lakes, its dissolved oxygen needs may be less than those of the zebra mussel.

Substrate Availability

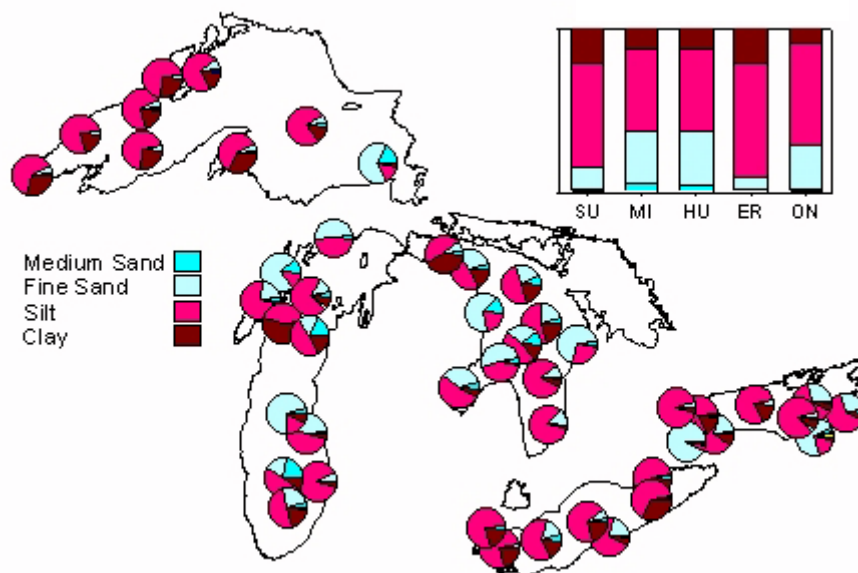
One of the most critical factors that affect the distribution and abundance of zebra mussels is substrate suitable for attachment. Juvenile and adult zebra mussels are epifaunal and generally sessile and are most abundant on rocky surfaces (Mellina and Rasmussen, 1994; Karatayev et al., 1998). The attachment of zebra and quagga mussels to hard substrates is a process that occurs when dreissenid post-veliger larvae search for their initial attachment location and with mobile adults. Under normal conditions over 99 percent of veligers do not reach a successful attachment stage. High mortality is expected for post-veligers unable to locate and settle upon suitable substrate (Stańczykowska, 1977). Post-veligers prefer substrate consisting of macrophytes, mussel aggregates, and pebbles (Lewandowski, 1982). Zebra mussels will colonize on any hard surface and can reach densities of up to 30,000 to 70,000 mussels per square meter (2,800 to 6,500 mussels per square foot) under certain conditions. Zebra mussels will also colonize soft, silty lake bottoms where harder objects are

deposited to serve as substrate (Ohio Sea Grant Program, 1995). However, preference for naturally occurring hard substrates may diminish over time as mussels become established in an area and juveniles colonize old shell. This can result in expansion onto adjacent soft substrates such as sand, mud, and gravel (Hunter and Bailey, 1992; Berkman et al., 2000; Czarnoeski et al., 2004).

In contrast, adult quagga mussels appear to be able to colonize both hard and soft substrates. They have formed extensive colonies on soft sediment in Lake Erie (Dermott and Munawar, 1993; Dermott and Kerec, 1997; Roe and MacIsaac, 1997). Quagga and zebra mussels have been found in western Lake Erie on soft substrates, displaying adaptation within 4 years of being introduced into the basin (Ohio Sea Grant Program, 1995; Berkman et al., 2000). In Lake Michigan they can colonize sand, clay, and pebbles, but not soft mud (Egan, 2006). As noted above, once a mussel is established on a hard or soft substrate, its shell can provide complex, hard substrate and promote colonization. Zebra mussels also will attach to one another, growing to thicknesses of up to 150 mm (6 in) (O'Neill, 1996).

The U.S. Environmental Protection Agency's (EPA) Great Lakes National Program Office reported the substrate composition of the Great Lakes for 1998 (EPA, 1998). (See Figure 1.) Silt and clay dominate the lakes, and Lake Michigan and Huron have the most sand. All substrate types in the Great Lakes could be colonized by quagga mussels because whereas substrate has been shown to affect population density and distribution, it has not been shown to restrict mussels from being present in systems due to their ability to colonize sand, mud, and hard substrate (Allen and Ramcharan, 2001).

FIGURE 1
Sediment Composition in the Great Lakes, Summer 1998



Source: EPA (1998).

Depth

Zebra mussels generally reach their highest densities in shallow water. Lake Ontario zebra mussel populations were most abundant at depths of 15 to 25 m (50 to 82 ft) (Mills et al., 1993). In Lake Erie, zebra mussels have expanded habitat into deeper, muddy substrate areas of the basin with an average depth of 10 m (33 ft) (Coakley et al., 1997). In Lake Ontario they have been reported at depths of 110 m (360 ft) (Mills et al., 1993).

In Lake Erie, zebra and quagga mussels coexist at depths of 8 to 110 m (26 to 360 ft). However, only quagga mussels are present at depths greater than 110 m (360 ft), as great as 130 m (425 ft) in Lake Ontario (Mills et al., 1993, 1999). Quagga mussels can thrive in both warm and near-freezing conditions of Lake Michigan, flourishing at depths of 300 ft and have been found as deep as 540 ft (Egan, 2006).

Colonization Effects

While low-density zebra and quagga mussel colonies may cause negligible impact, high-density colonies have led to major ecological and economic problems since their arrival in North America. Both species are prodigious water filterers, removing substantial amounts of phytoplankton and suspended particulates from the water. By removing the phytoplankton, dreissenid in turn decrease the food source for zooplankton, therefore altering the food web (USGS, 2007a, b). USGS (2007a) summarized studies showing the decreases of plankton due to large dreissenid colonies reducing zooplankton biomass through reducing phytoplankton. (See Table 2.) Zebra mussels filter small particles 90 percent more efficiently than native unionid bivalve mollusks, and dreissenid infestations have decreased unionid populations (Nalepa, 1994; USGS, 2007a). A study by the National Oceanic and Atmospheric Administration's (NOAA) Great Lakes Environmental Research Laboratory found that zebra mussels also promote and maintain *Microcystis* blooms, a potentially toxic blue-green alga, by filtering *Microcystis* out of water but eating other algae, *Microcystis*'s competitors (Vanderploeg et al., 2001).

TABLE 2
Summary of Studies Reporting Phytoplankton Decline due to Large-Scale *Dreissena spp* Invasions*

Location	Effects after <i>Dreissena spp</i> invasions	Reference
Lake Erie	Diatom declined 82–92%	Holland (1993)
	Total algae declined 62–90% from 1988 to 1990	Nichols and Hopkins (1993)
	Zooplankton declined 55–71%	MacIsaac et al. (1995)
Saginaw Bay, Lake Huron	Chlorophyll- <i>a</i> declined 60–70%; zooplankton decreased 40% from 1991 to 1992	Fahnenstiel et al. (1993)
Hudson River	Phytoplankton biomass declined 85%; zooplankton declined 70%	Caraco et al. (1997)

* From USGS (2007a) data.

Dr. Thomas Nalepa with NOAA reported that Lake Huron alewives, smelt, and bloater populations, which feed on zooplankton, have suffered greatly owing to the invasion of quagga, which severely decrease food availability for the larger fish that prey on these smaller fish. Nalepa also stated that Michigan's coho and chinook salmon stocking rates

were reduced by 50 percent in response to mussels' negative impact on food availability (AP, 2007).

In addition to decreasing chlorophyll-*a*, the filtration of water is associated with increases in water transparency and accumulation of pseudofeces (Claxton and Mackie, 1998). Increased water clarity enhances light penetration, causing a proliferation of aquatic plants that can change species dominance. This alters entire ecosystems and creates viable substrate from plants for veligers to expand colonies. Increased water clarity can also alter thermoclines by increasing water temperature. The accumulating pseudofeces produced by high-density dreissenid colonies create a polluted environment (USGS, 2007a). The process of waste decomposing depletes oxygen, creates acidic conditions, and produces toxic byproducts (USGS, 2007b). In addition, quagga and zebra mussels accumulate organic pollutants within their tissues to levels more than 300,000 times greater than concentrations in the environment, and these pollutants are found in their pseudofeces. These bioaccumulated toxins can be passed up the food chain, thereby increasing wildlife exposure to organic pollutants (Snyder et al., 1997; USGS, 2007a).

Another major threat from high *Dreissena spp* density involves the fouling of native freshwater mussels. In addition to competing for food, zebra mussels are known to heavily colonize any hard substrata, including native mussels and other invertebrates. This can cause stress and even mortality due to feeding interference, and this fouling has severely reduced populations of native mussels.

High *Dreissena spp* density can also change habitat for other species. The *Dreissena spp* beds negatively affect blue gill, a major Great Lakes fisheries species, by decreasing their predation rates on amphipods by providing amphipods spatial refugia (González and Downing, 1999). Similar decreased foraging efficiency was reported with native mottled sculpin (*Cottus bairdi*) (McCabe and Marsden, 2001).

The ability to rapidly colonize hard surfaces causes serious economic problems. These major biofouling organisms can clog water intake structures, such as pipes and screens, therefore reducing pumping capabilities for power and water treatment plants, costing industries, companies, and communities. Recreation-based industries and activities have also been affected; docks, breakwalls, buoys, boats, and beaches have all been heavily colonized (USGS, 2007a).

Potential *Dreissena spp* colonization impacts are not completely clear owing to the relatively short time span of their presence in North America. However, it is certain from studies thus far that *Dreissena spp* have a high potential for rapid adaptation leading to significant long-term impacts in North American waters (Mills et al., 1996).

Population Distribution

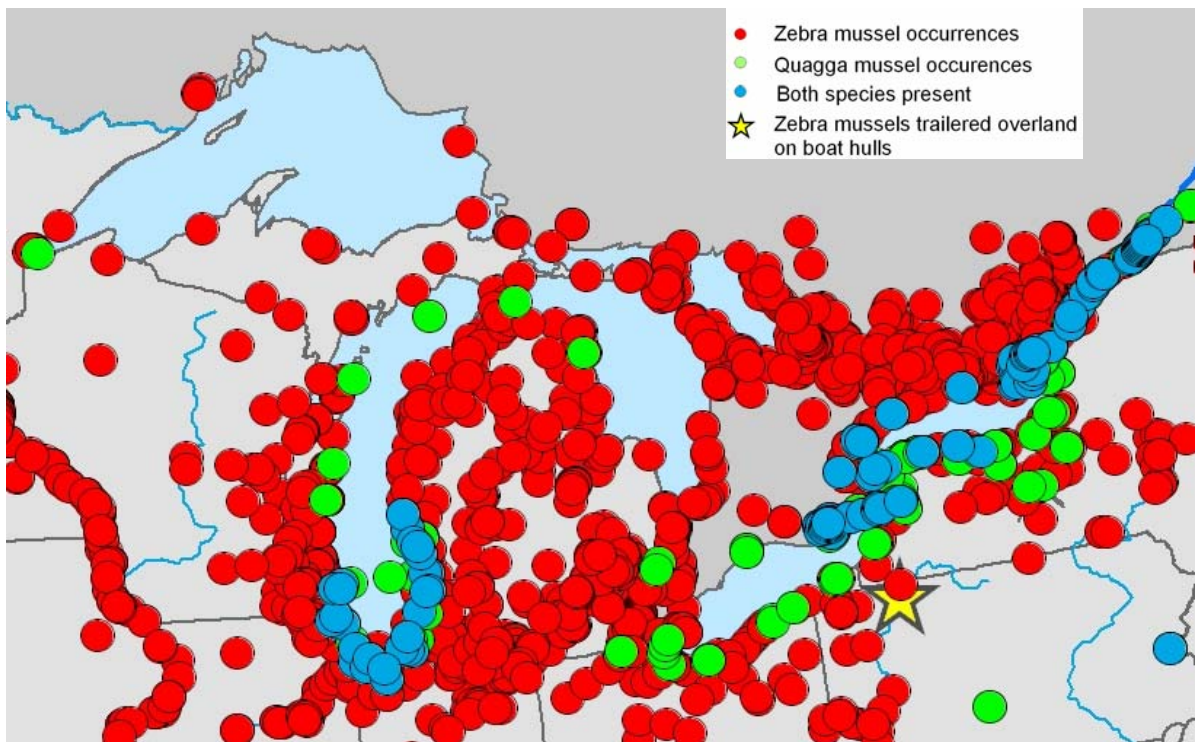
A population shift has occurred within the *Dreissena spp* since the early 1990s. The large shell size and low respiration rates of quagga mussels are competitive advantages against the zebra mussel and may explain their increasing dominance between the two species (Stoeckmann, 2003). In 1992, quagga mussels greatly outnumbered zebra mussels only in the eastern basin of Lake Erie, but now the entire lake is dominated with quagga mussels (Mills et al., 1993; Patterson et al., 2002). Additionally, Patterson et al. (2002) reported that the *Dreissena spp* basin-average, shell-free dry tissue mass in Lake Erie increased nearly

fourfold from 1992 to 2002. Quagga mussels dominate the *Dreissena spp* in nearshore regions of Lake Ontario as well (Wilson et al., 2006).

Currently, Lake Superior does not have a large *Dreissena spp* invasion. No quagga mussels were observed in Lake Superior in a 2002 survey; however, they were observed in 2005 and in 2007 as expected owing to their ability to spawn at temperatures lower than zebra mussels can and survive with a lower food supply (Grigorovich et al., 2003; EPA, 2007; USGS, 2007a). The current area of reproduction is in the Duluth-Superior harbor (EPA, 2007; Minnesota Sea Grant, 2007). Doug Jenson (personal communication, 2007) with the Minnesota Sea Grant attributes the isolated harbor colonization to the harbor's being less influenced by Lake Superior and by having shallower, warmer waters with higher calcium levels. Jenson also commented that despite the large magnitude of larva floating from the Duluth-Superior harbor into the western basin, no massive colonies exist in the larger lake. Due to Lake Superior's low calcium levels, Doug Jenson (personal communication, 2007) and Thomas Nalepa (AP, 2007) do not believe quagga mussel colonization will be as large scale as in the other Great Lakes.

CH2M HILL investigated the existence of up-to-date, open-water population density maps for all the Great Lakes through literature searches and personal correspondence with federal, state, and university authorities (Benson, 2007; Bunnell, 2007; Kreiger, 2007; Mayer, 2007; Mackey, 2007; Ciborowski, 2007). All resources concluded that due to the expansive scope of such a study and insufficient funding, no recent open water *Dreissena spp* distribution maps exist for the entire Great Lakes. The U.S. Geological Survey (USGS) has produced a nearshore map (see Figure 2) displaying the presence of quagga and zebra mussels in the Great Lakes for 2007 but not showing open water information or density values (USGS, 2007c). Benthic surveys performed annually by EPA can provide only mussel presence or absence data due to the provisional characteristics of the *Dreissena spp* portion of the study (EPA, 2007). (See Figure 3.)

FIGURE 2
Map of *Dreissena spp* Nearshore Distribution for 2007



Source: USGS (2007).

However, maps showing open-water distribution patterns in Lake Erie and south Lake Michigan were created for this report. To further investigate the distribution patterns of Lake Erie and south Lake Michigan, basin bathymetry and cargo ship sweep lines were included in the figures. The zebra and quagga survey maps highlight the 10-m and 100-m contours according to their respective depth preference, as previously discussed (Mills et al., 1993, 1999; Egan, 2006). The cargo ship sweep lines were produced from data from USCG (2005).

Lake Erie quagga and zebra mussel distribution maps (see Figures 4 and 5) were created using data from an environmental monitoring and assessment program (Ciborowski et al., 2007). Depth is not a limiting factor for the quagga mussels in Lake Erie because the maximum depth is 210 feet, within the quagga mussel preference. These figures display the dominance of quagga mussels over zebra mussels in Lake Erie and reflect the limiting effects of anoxia on dreissenid colonization reported by Dermott and Munawar (1993). Lake Erie's central basin area with periodic summer anoxia was the only region that was not colonized with *Dreissena spp*. Potential areas of concern may be areas to the east and west of this absence region, where sweeping and *Dreissena spp* presence was reported. However, because *Dreissena spp* are present throughout the lake, dry cargo residue discharged here may not promote increased *Dreissena spp* colonization any more than the existing colonies themselves promote colonization by creating their own substrate.

The southern Lake Michigan *Dreissena spp* distribution maps (see Figures 6 and 7) were created using data from NOAA (Nalepa, unpublished data, 2004. As in Lake Erie, quagga

dominance is reflected here as well. A potential area of concern in southern Lake Michigan is the open water east of Chicago, where sweeping was reported. Depth is not a limiting factor in this area owing to its being less than 100 m (300 ft), and quagga mussel presence was confirmed at the sites. Any additional hard substrate here may promote increased *Dreissena spp* colonization. Near shore localized anoxia is possible in Lake Michigan and may account for the absence of *Dreissena spp* near Michigan City (Bunnell, 2007).

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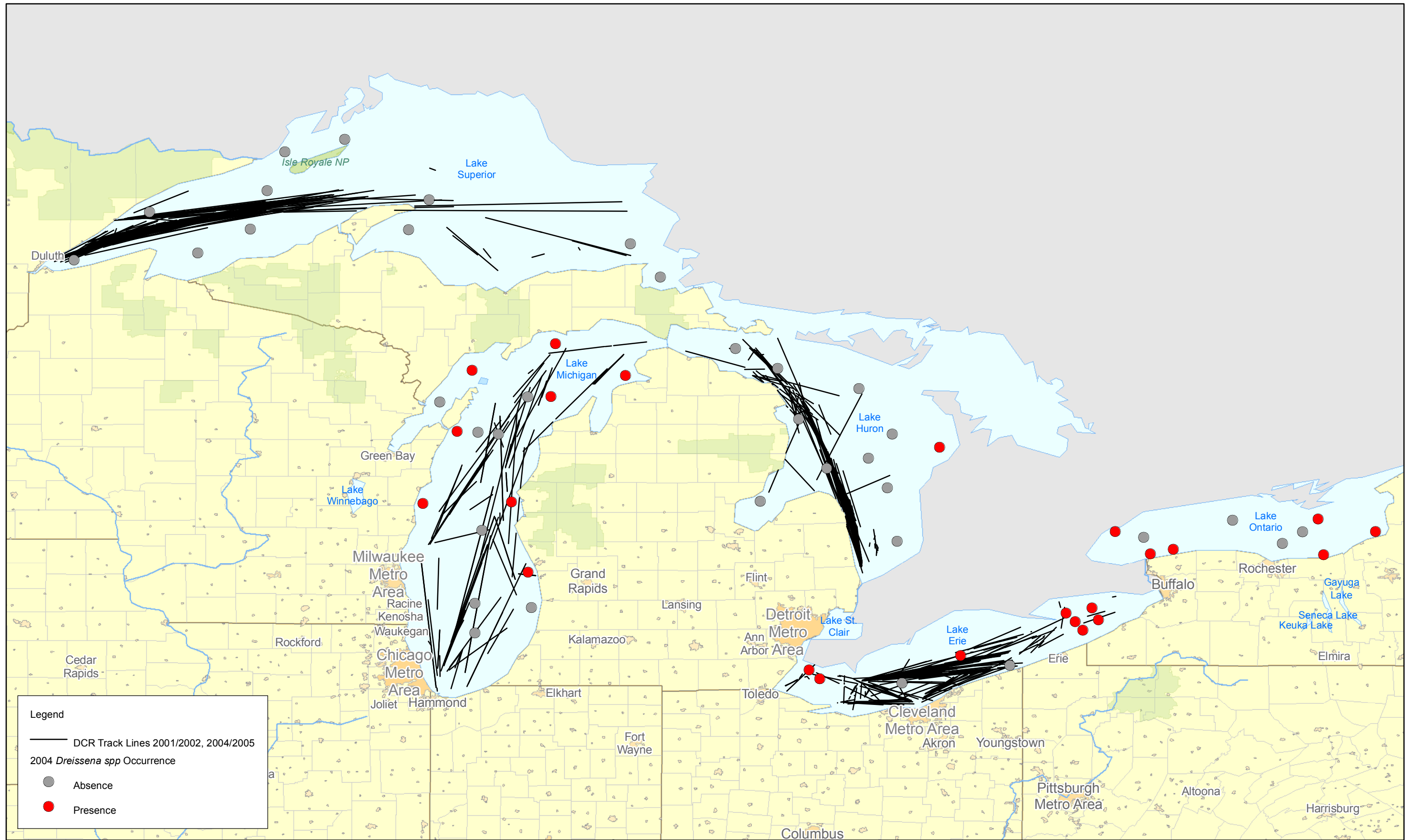


Figure 3
Dreissena spp, EPA Benthic Survey Stations
 Created by CH2M HILL from EPA Great
 Lakes National Program Office 2004 benthic data

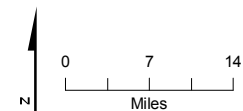
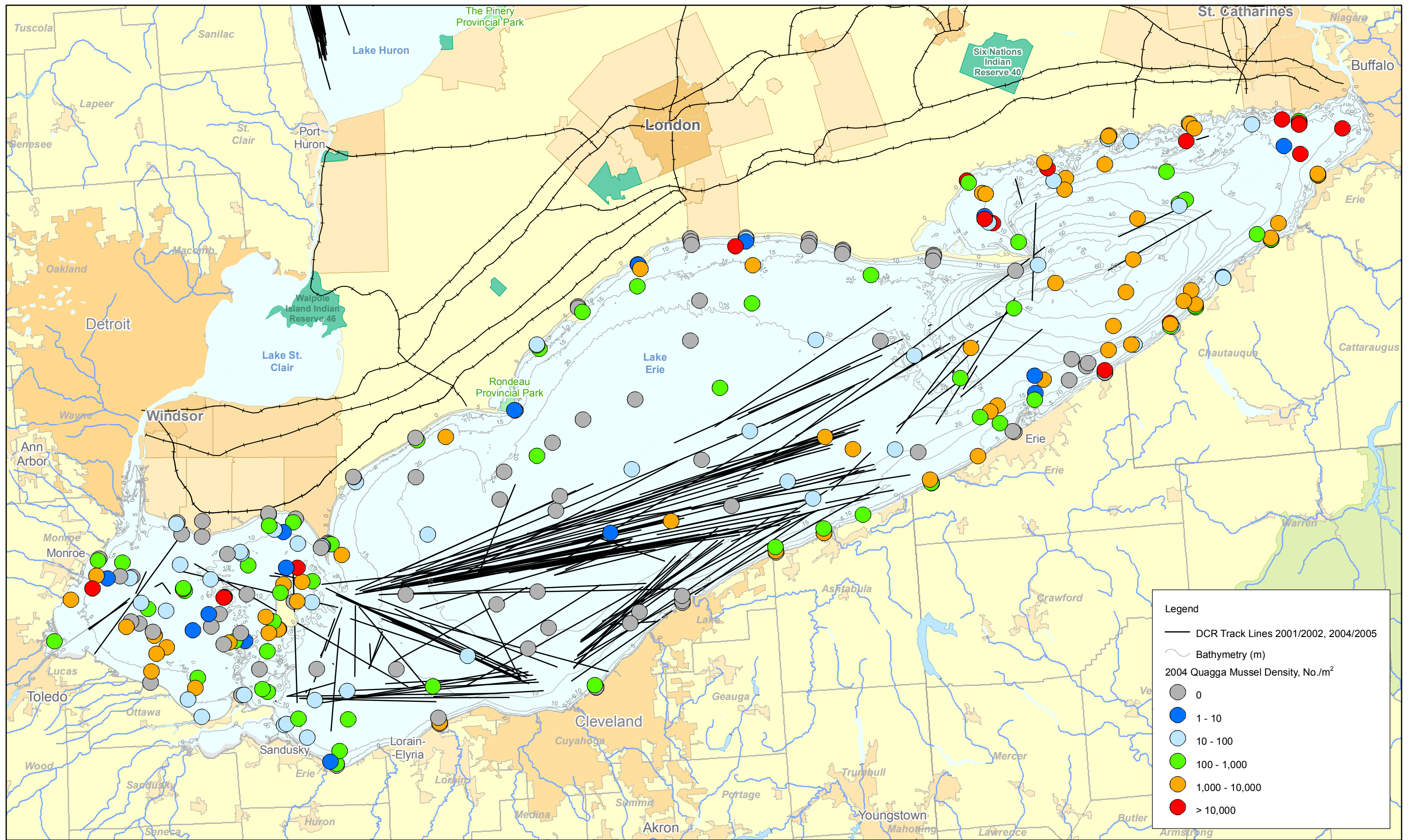


Figure 4
USCG Great Lakes Mussel Report
Created by CH2M HILL from Ciborowski, J. H., D.R. Barton, et al. (2007) data

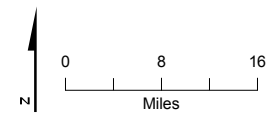
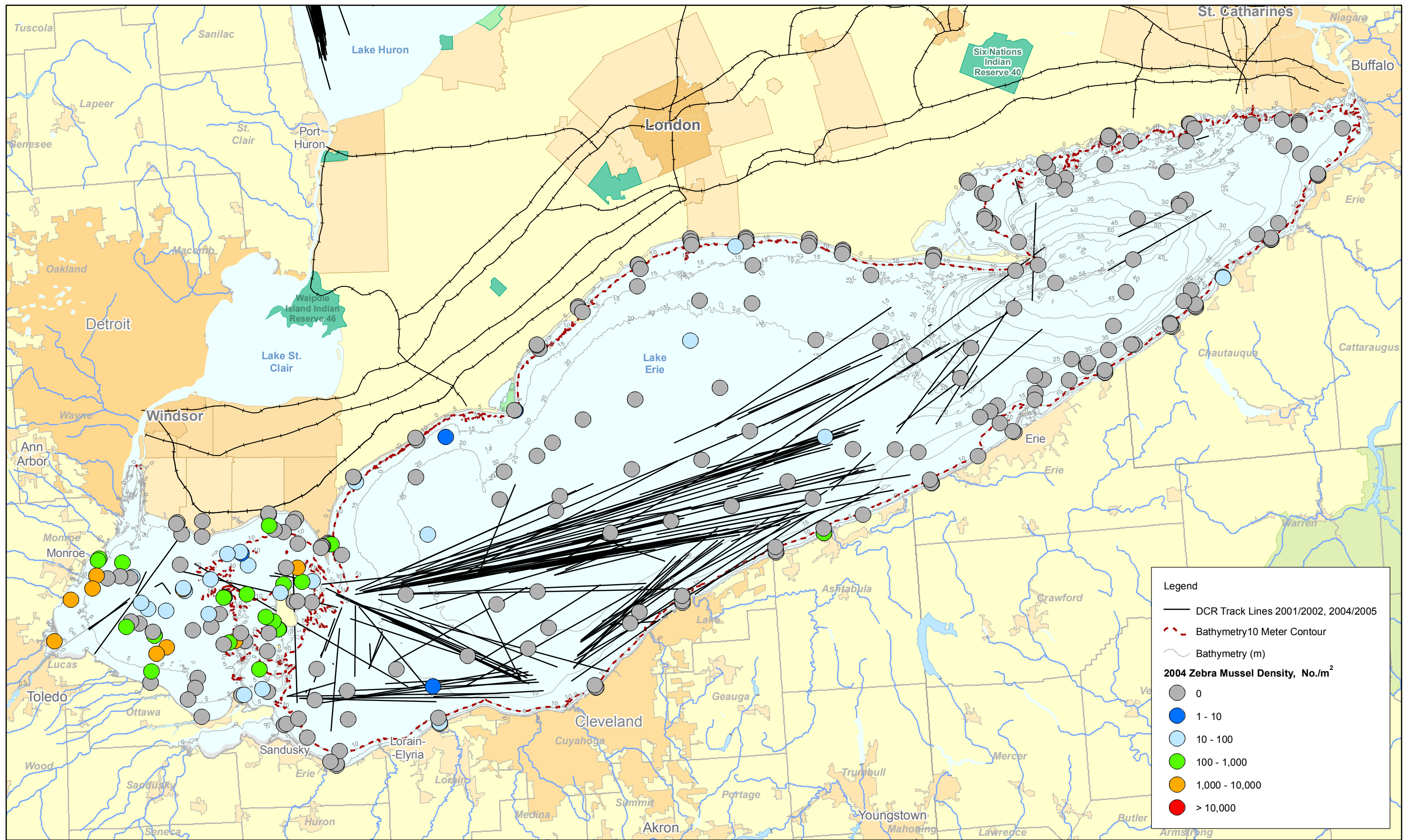


Figure 5
USCG Great Lakes Mussel Report
Created by CH2M HILL from Ciborowski, J. H., D.R. Barton, et al. (2007) data



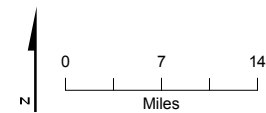
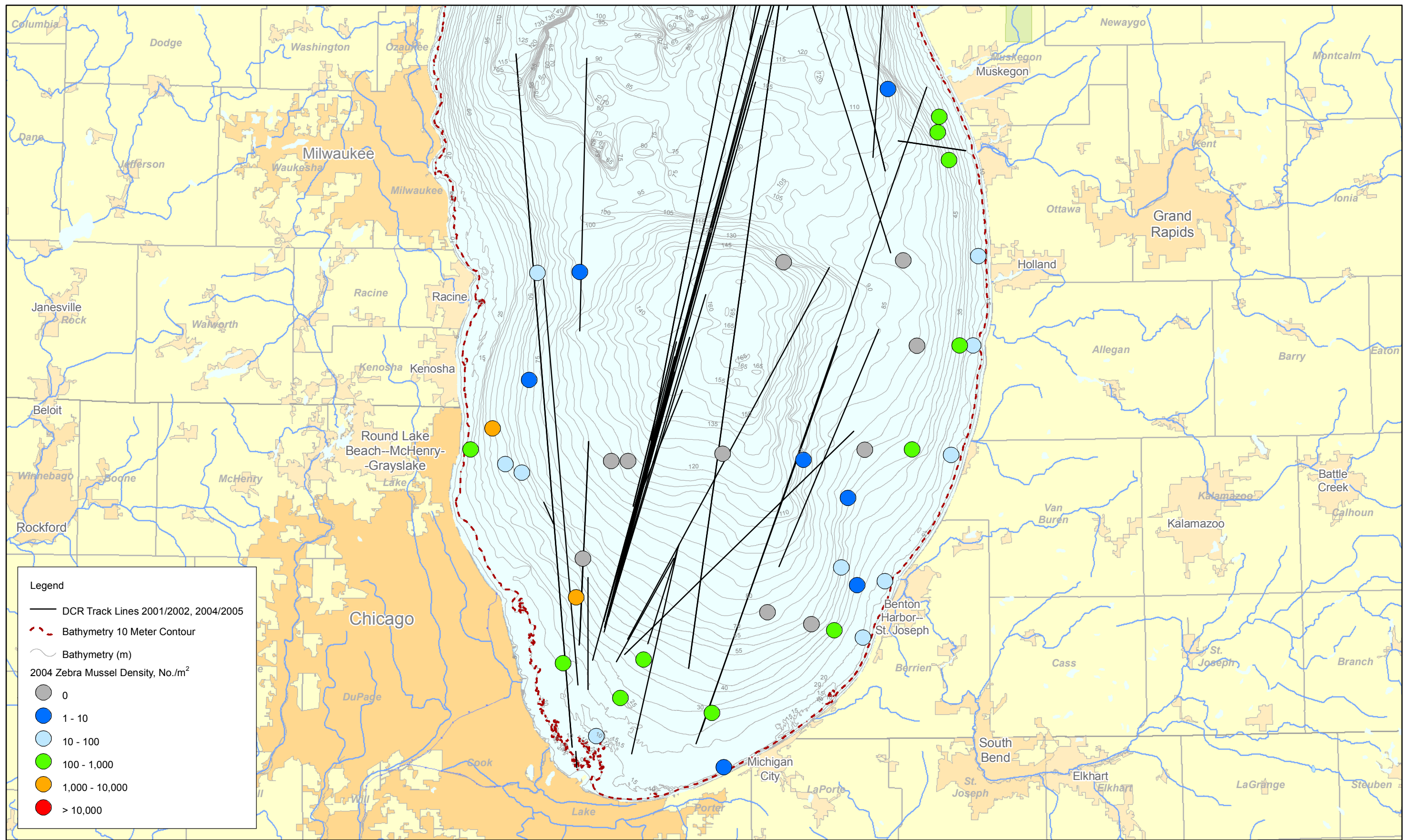


Figure 7
USCG Great Lakes Mussel Report
Created by CH2M HILL from Nalepa, T., unpublished data